THE POLLINATION ECOLOGY OF *SCLEROCACTUS BREVIHAMATUS* SSP. *TOBUSCHII* (CACTACEAE) IN THE EDWARDS PLATEAU REGION OF WEST-CENTRAL TEXAS

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ABSTRACT

The Tobusch fishhook cactus (*Sclerocactus brevihamatus* ssp. *tobuschii*) (Cactaceae) is an endangered species endemic to the Edwards Plateau region of west-central Texas. This study's objectives were to provide information on pollination ecology by comparing pollinator effectiveness among floral visitors and determining the breeding system and floral attraction system. Research was conducted on two field sites located in Kimble and Kerr Counties and on greenhouse specimens from Val Verde and Edwards Counties during the reproductive period (February-July) of 2014 and 2015. Honey bees (Apidae) were determined to be the most frequent and effective pollinators, followed by halictid bees (Halictidae). Fruit set results from pollination treatments and pollen-ovule (P/O) ratios support the reports from previous studies that this species is primarily self-incompatible. The flowers are protandrous with anther dehiscence occurring in the bud. Lastly, pollen was determined to be the primary floral reward.

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INTRODUCTION

Sclerocactus Britton & Rose (Cactaceae) is a relatively small genus containing approximately 22 species. There has been considerable controversy concerning the taxonomy of this genus, with some researchers including *Ancistrocactus*, *Echinomastus*, *Glandulicactus*, and *Sclerocactus* as a single genus whereas others separate them (Porter et al. 2012). These taxa primarily occur in dry highland deserts throughout the southwestern part of the United States, including California, Arizona, Nevada, New Mexico, Utah, Colorado, and Texas (Hochstätter 1995). Plants are characterized by solitary, rarely branching, ribbed stems with tubercles 6-12 mm long which are covered with hooked spines arising from the areoles. Flowers are primarily campanulate or funnelform and can be a range of colors, including pink, purple, and yellow. Fruits are ovate to cylindrical with fleshy, reddish-to-greenish coloration (Heil and Porter 1994). Many members of this genus are considered either threatened or endangered (Porter et al. 2012). Included in the protected group is the endangered Tobusch fishhook cactus (*Sclerocactus brevihamatus* ssp. *tobuschii*), the focus of this study.

The Tobusch fishhook cactus was listed by the US Fish and Wildlife Service as endangered on November 7, 1979, and the state of Texas listed the species as endangered on April 29, 1983 (USFWS 2010). This taxon is endemic to the Edwards Plateau region of west-central Texas, occurring in Bandera, Edwards, Kerr, Kimble, Kinney, Real, Uvalde, and Val Verde Counties (Fig.1) where it grows on thin, rocky soils underlain by limestone usually in open areas of oak-juniper or oak-pine woodland (Sutton et al. 1997, Brown and Best 2008). One of the main threats to its conservation is habitat modification. Although urban and

industrial development in the area is sparse, subdivisions of privately-owned land into smaller “ranchettes” have increased the amount of roads, fences, power lines, and residential development which have contributed to habitat fragmentation (USFWS 2010). Another primary threat is the Tobusch weevil, an undescribed species of weevil (*Gerstaeckeria* sp.) which parasitizes the Tobusch fishhook cactus, its preferred host plant. The Tobusch weevil has contributed to significant declines in many known populations (USFWS 2010).

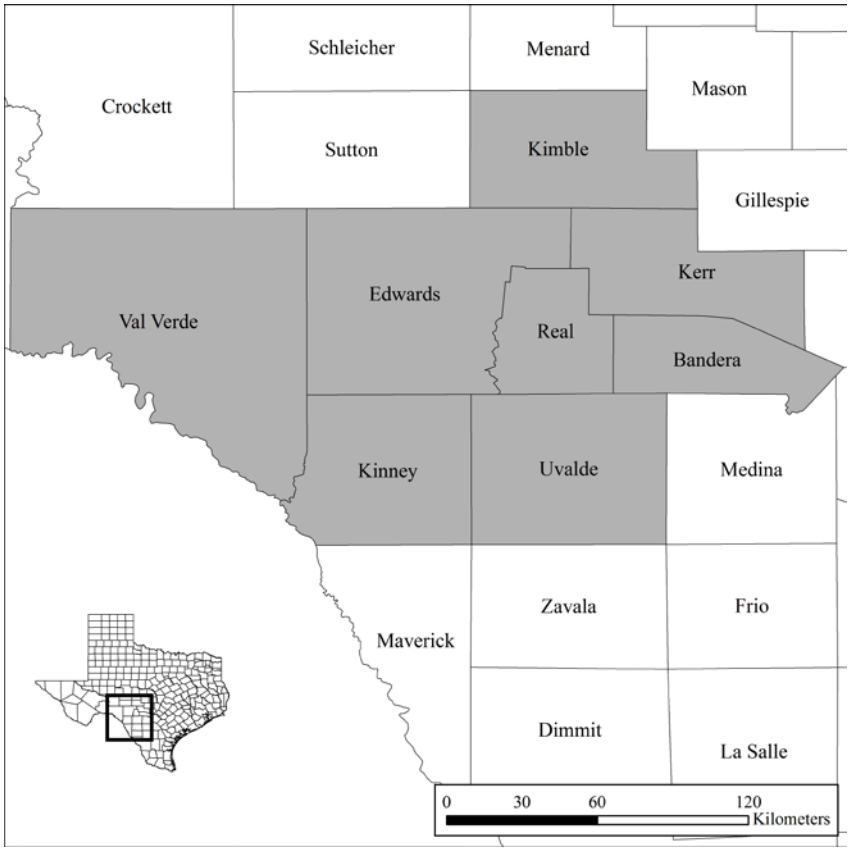

FIG. 1—Map of Texas counties with known populations of the Tobusch fishhook cactus (*Sclerocactus brevihamatus* ssp. *tobuschii*).

Concerns over the conservation of the Tobusch fishhook cactus have prompted research into the reproductive biology of this species, including studies on pollination

ecology and breeding systems. Much of the reproductive and pollination ecology known about the Tobusch fishhook cactus is from Ray Emmett's doctoral dissertation (1995), in which he investigated pollination, seed ecology, mortality, reproduction, and growth in three populations. Emmett conducted his studies at the Walter Buck Wildlife Management Area and the Devil's Sinkhole State Natural Area from 1991 to 1994. His third study site was at the Kickapoo Caverns State Natural Area where he conducted studies from 1993 to 1994 (Emmett 1995). According to a report from the Texas Parks and Wildlife Department (TPWD 1984), Tobusch fishhook cacti are fast growing and short-lived, reaching reproductive maturity within three years and not living past thirteen years. Poole and Janssen (2002) reported that reproduction begins once individuals reach 20 mm in diameter, and plants can live up to 10 or more years. However Emmett, by measuring stem diameter growth, determined that it would take approximately 9 years to reach reproductive maturity and 25 years to reach a modest diameter of 30 mm.

The only reported method of reproduction for this taxon is through sexually produced seeds (TPWD 1984). The Tobusch fishhook cactus flowers only once per year from early February to mid-March (Emmett 1995). Poole and Janssen (2002) observed that the annual flowering period is 2-3 weeks, beginning as early as late January in the southern part of the range, and as late as mid-March in the northern part of the range. The flowers are yellow-green and arise from the previous year's tubercles. The larger plants can have up to eight or more flowers at one time. The flowers are diurnal and only open during periods of adequate sunlight and warmth, and the flowers can reopen daily for up to a week or until pollinated. The fruits (2-3 cm in length) become mature in mid to late May (Emmett 1995). The fruit dries and splits longitudinally, carrying an average of 20-40 papillate black seeds (1.5 mm

long, 1.5 mm broad, and 1 mm thick) per fruit (Benson 1982). An earlier report of self-incompatibility from Zimmerman in 1981 was better defined in controlled pollination experiments performed by Emmett (1995). He found that cross-pollinated flowers had 98% fruit set and an average of 38.9 seeds per fruit, while self-pollinated flowers only had 5% fruit set and 1.1 seeds per fruit. The germination rate was also greater in cross-pollinated seeds (22.7%) compared to self-pollinated seeds (6.3%).

Emmett also tested how the proximity of pollen donors affected fruit set, seed set, and seed germination by comparing the effectiveness of crosses using pollen collected from neighboring plants and distant plants; he found no significant differences. Since Tobusch fishhook cacti have been shown to be primarily self-incompatible, the similar pollination effectiveness between neighboring and distant plants suggests there is adequate genetic diversity between local populations (Emmett 1995).

Studies by Emmett (1995), Lockwood (1995), as well as Reemts and Becraft (2013) have identified a number of insect species as potential pollinators. Table 1 summarizes the insect visitors for four different sites. Even though there is much variability between sites in regard to floral visitors, honey bees and halictid bees are frequent visitors to all three studies. Although floral visitors were recorded in these studies, it is still unknown which are effective pollinators.

TABLE 1--Compilation of insects collected from four studies of the Tobusch fishhook cactus. (BWM = Walter Buck Wildlife Management Area (Kimble County) from 1991 to 1994, Emmett (1995); DSH = Devil's Sinkhole State Natural Area (Edwards County) from 1991 to 1994, Emmett (1995); KPC = Kickapoo Caverns State Natural Area (southern Edwards County and northern Kinney County) from 1992 to 1994, Lockwood (1995); LCP = Love Creek Preserve (Bandera County) 2013, Reemts and Becraft (2013)).

Family	Species or Description	BWM	DSH	KPC	LCP
Apidae	*Anthophora californica*				X
Apidae	*Apis mellifera*	X	X	X	X
Halictidae	*Agapostemon texanus* or *angelicus*		X		X
Halictidae	*Agapostemon* sp.			X	
Halictidae	*Lasioglossum semicaeruleum*	X	X	X	X
Halictidae	*Lasioglossum morilli*		X	X	
Halictidae	*Lasioglossum* (*Dialictus*) *connexum*	X	X		
Halictidae	*Lasioglossum* (*Dialictus*) sp.	X	X		
Halictidae	*Lasioglossum* (*Dialictus*) *comulum*			X	
Megachilidae	*Osmia subfasciata*			X	
Chrysomelidae	*Diabrotica undecimpunctata*		X	X	X
Formicidae	Small black ants			X	
Lycaenidae	Hairstreak butterfly				X
Moredellidae	Tumbling flower beetle				X
Phoridae	Scuttle flies				X
Pieridae	*Eurema nicippe*			X	
Pieridae	Sulphur butterfly				X
Syrphidae	*Toxomerus marginatus*		X		
Uncertain	Small, red mites				X

Emmett (1995) investigated seed ecology including dispersal and predation, annual seed germinability, period of germination, and germination success. At each of the three sites, a species of ant (*Forelius foetidus*) (Dolichoderinae), removed up to 85% of the seeds from most fruits and transported seeds to the mound, although seeds do not appear to have an elaiosome or caruncle. The fate of seeds removed by ants is currently unknown. For the

remaining 15-20% of seeds, dispersal was mainly by rainwater or gravity, which appears to explain the large number of seedlings near mature plants.

There were three main objectives to this study. The first objective was to identify effective pollinators by first determining stigma receptivity and pollen viability. Once stigma receptivity and pollen viability were understood, the importance and efficiency of flower visitors were evaluated by comparing intrafloral behavior, time of flower visitation, average duration of visit, and overall abundance. Stigma receptivity is a crucial stage in reproduction. It can greatly influence pollination success at different stages in a flower's life and the relative importance of floral visitors. In order to be an effective pollinator, the pollinator must be present at the time of stigma receptivity and must also transfer viable pollen to the receptive parts. In addition, assessment of pollen viability is critical in understanding pollen-stigma interactions and evaluating pollen flow in a population. The second objective of this study was to determine the extent of self-compatibility. Knowing the level of self-compatibility in a species is essential for conservation efforts because it determines what extent of genetic diversity is necessary to maintain a viable population. The last objective of this study was to access the reward system used to attract potential pollinators.

METHODS

Study Site

This study was conducted during the reproductive period (mid-February through July) in 2014 and 2015 at two field sites that support naturally occurring populations. The first site is in Kimble County on a highway median along I10 east of Junction with a 2012 estimate of 308 individuals (J. Poole, pers. comm.). The second site is located approximately 87 km southeast of the Junction site at the Kerr Wildlife Management Area (Kerr WMA) in Kerr County and was estimated in 2015 to contain approximately 670 individuals (J. Poole, pers. comm.). In addition, 104 Tobusch fishhook cactus plants from the UT-Lady Bird Johnson Wildflower Center (Austin, Texas) were used for greenhouse studies involving stigma receptivity and pollen viability. One hundred of these plants were originally harvested from a pipeline project in Val Verde County (Fig. 2), and four were harvested from a pipeline project in Edwards County.

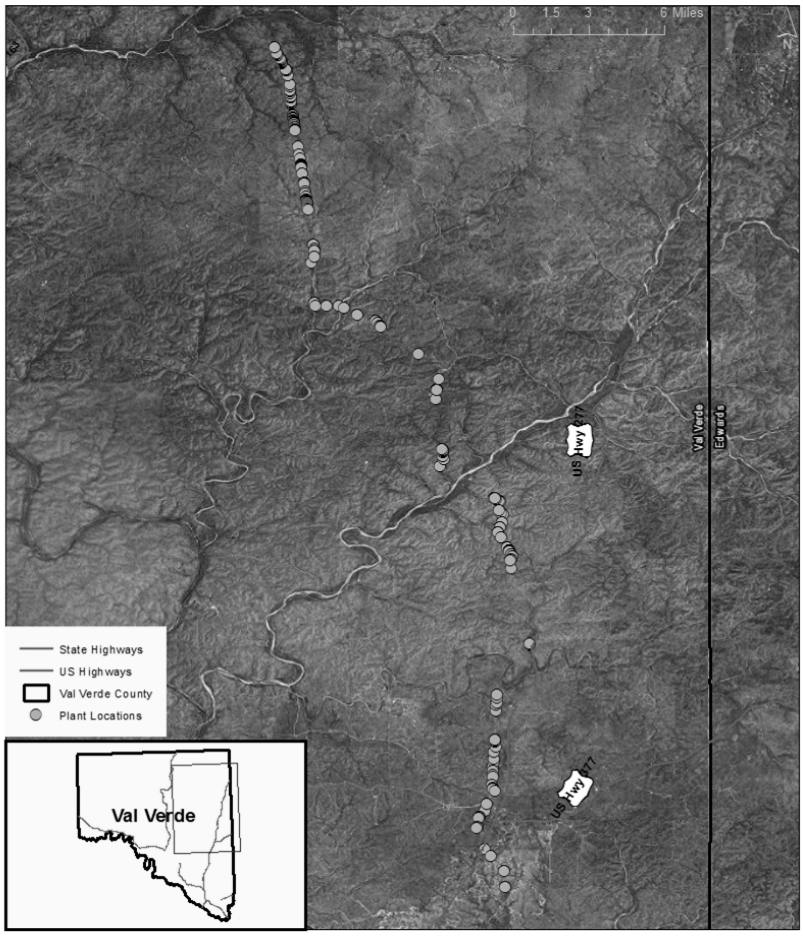

FIG. 2--Map of GPS locations of 100 Tobusch fishhook cactus plants harvested prior to a pipeline construction project in Val Verde County.

Both field sites and the original site of the greenhouse plants are located on the Edwards Plateau in central Texas which spans approximately 150 km north to south and is characterized by thick, flat bedrock layers composed primarily of Cretaceous limestone (Fowler 2005). Elevation on the Edwards Plateau ranges from 0.2-0.9 km above sea level. Mean annual temperature for the region is approximately 21°C with an average low of 4°C in January and an average high of 36°C in August (Fowler 2005). Average annual precipitation varies widely from 58-89 cm per year, with the eastern part of the region receiving more rainfall than the western part of the region (Texas A&M Forest Service 2014).

Floral Phenology

Stigma Phenology and Receptivity

Stigma phenology was determined by noting stigma lobe positions and color in open flowers of different ages. In addition, images taken of flowers over a period of several days with Wingscapes® TimelapseCam-WCT-00122 (Alabaster, AL) cameras were used to observe how the appearance of the stigma changes over the life of the flower.

Timing, receptive area, and duration of stigma receptivity were determined using Peroxtesmo esterase indicator papers (two papers + 1 ml water) (Dafni and Maués 1998). For this test, indicator papers were placed on the stigmas (N=37) harvested from flowers that had been previously covered with insect exclusion cages in order to prevent insect visitation. The solution causes the receptive portions of a stigma to change from yellow/green to a dark blue color. For this study, presence of any staining was considered evidence of receptivity.

Stamen Phenology and Pollen Viability

Anther dehiscence and stamen positioning was determined through field observations of open flowers of varying ages and dissection of unopened mature flower buds. Images of individual flowers over several days using time lapse cameras were used to determine daily changes in stamen features.

In 2014, 18 flowers were collected in order to compare pollen viability between young (N=6) and mature (N=12) flowers. Age of the flower was determined by the color of the stigma and position of the stigma lobes. Two staining techniques were used to detect viable pollen: the thiazolyl blue tetrazolium bromide (MTT) technique (Hecker 1963) and the diaminobenzidine (DAB) test for peroxidases (Dafni et al. 2005). Results of tests were compared with a control group consisting of killed pollen. Pollen was killed by baking it in

the oven at 93ºC for either 2 hours or 24 hours. Results showed that the DAB test stained both 2 hr. and 24 hr. killed pollen while the MTT staining technique did not. Therefore, only the MTT staining technique was used to determine pollen viability during the 2015 field season. In 2015, 10 young flowers and 27 mature flowers (N=37 total) from caged plants were collected to estimate pollen viability by flower age. For this technique, pollen was placed in a test tube and stained with 0.5% tetrazolium bromide solution in 40% sucrose solution. Pollen remained in the stain for approximately 2-5 minutes and then the solution was vortexed for 30 seconds. The pollen was then pipetted on to a Neubauer Improved DHC-N01 C-Chip disposable hemocytometer (DigitalBio, Seoul, South Korea) and the protocol described in the instructions manual was used to count viable and nonviable pollen. The pollen was considered viable if it stained purple-deep purple, while pollen was considered non-viable if it did not stain at all.

Nectar Study

During the 2014 study, 4 techniques were employed to test for the presence of nectar. For each test, plants with flower buds were covered with insect exclusion cages to prevent nectar removal and contamination by insects upon anthesis. Nectar wicks (7x3 mm) were made using 100% rag cotton paper and a point punch. The wicks, held with forceps, were inserted into the base of freshly collected flowers in an attempt to saturate the wicks with nectar. If no saturation occurred within a minute the flower was considered to be dry. In addition to the nectar wicks, glucose reagent test strips (Diastix®), also held with forceps, were inserted into the base of the flower for approximately 15 seconds and then the strip checked for a color change. For further analysis, flowers (N=29) were transported to the lab and tested with either 1:10,000 neutral red (N=19) (Jürgens et al. 2009) or phenol/sulfuric

acid (N=10) (Schemske 1978). For the neutral red test, harvested flowers were cut in half longitudinally, and immediately submerged in a 1:10,000 neutral red solution (0.1g of stain in 1 L water). After approximately 20 minutes, flowers were removed from the stain, rinsed in dH_2O to remove excess stain and the flowers were observed under a dissecting microscope for stained areas. Areas that stain red indicate the presence of active floral nectaries. For the phenol/sulfuric acid test, dH_2O was used to rinse potential nectar from the inner base of the flower. The rinse water was then treated with 4-5 drops of concentrated H_2SO_4 followed by 2-3 drops of 5% phenol solution. In the presence of carbohydrates the solution becomes yellow-gold in color. Neutral red staining (N=11) and the phenol/sulfuric acid method (N=10) were repeated during the 2015 study with 21caged flowers.

Some species of cacti also produce extrafloral nectar by glands located in the tubercle-grooves (Elias 1983). A clear, sticky substance was observed being produced at the base of the tubercles from greenhouse specimens during the 2014 fruiting period. The substance was collected (N=6) by placing wicks held by forceps on the extrafloral nectary until saturated. Saturated wicks were then placed in a micro centrifuge tube, transported to an ultracold freezer, and stored at –50°C until analyzed for the presence of nectar using glucose reagent test strips (Diastix®).

Pollen-Ovule Ratio

Cruden (1977) proposed that pollen-ovule (P/O) ratios can be used as good indicators of the species' breeding system and also gives insight on the efficiency of which pollination occurs (i.e. the likelihood of a pollen grain reaching the stigma). According to Cruden (1977), plant species with xenogamous breeding systems will, in general, have higher P/O ratios than autogamous species. In order to determine P/O ratios, 9 unopened mature buds

were collected from the field. The buds were cut in half longitudinally with a razor blade. The stamens were removed from the flower and placed evenly on a grid under a dissecting microscope, and the number of anthers counted. Next, the grid was cleared, all ovules were removed from the ovary and placed evenly throughout the grid, and the number of ovules counted. The average number of pollen grains of 4 anthers per flower was counted using a Neubauer Improved DHC-N01 C-Chip disposable hemocytometer (DigitalBio, Seoul, South Korea). The average was multiplied by the number of anthers to estimate the total number of pollen grains per flower. The total number of pollen grains was divided by the number of ovules to determine the P/O ratios for each flower.

Flower Anthesis

To determine the effects of temperature and cloud cover on flower anthesis, 9-10 time lapse cameras, focused on flowers, were set to capture an image every 10 seconds from 7am-7pm for a total of 12 days from February-April of the 2015 field season. However, on 2 of the 12 days the flowers never opened. A total of 53,085 images were taken of open flowers. The opening/closing times were recorded for each day; flowers were considered open when the reproductive parts (i.e. stamens and pistil) became visible and considered closed when they were no longer visible. Hourly temperatures (°C) and cloud cover data were obtained from the [ERV] Kerrville Municipal station of the TX_ASOS network on the Iowa Environmental Mesonet website (IEM 2015). A logistic regression analysis using an additive model including the variables date, hour, temperature (°C), and cloud cover types was used to identify factors affecting floral anthesis.

Evaluating Pollinator Effectiveness

After stigma phenology and pollen viability were determined, effective pollinators were identified by intrafloral behavior (i.e. contact with the stigma), time of flower visitation (when stigma was receptive), average duration of visit, and overall abundance throughout the flowering season. The frequency of each floral visitor through the flowering season was calculated by counting the number of images containing each type of floral visitor; each image is defined as an observation. Average floral visitation time was estimated by counting the number of images an individual insect visited the flower without leaving. Since images were taken at 10 second intervals, a 5 second estimate was used for the first and last observation.

Breeding System Investigation

Prior to hand-crossing experiments, reproductively mature plants (2014: N=41; 2015: N=35) were covered with pollination exclusion cages (a wire mesh ring covered with bridal veil (mesh size: 1x1 mm)) to ensure virgin flowers. In 2015, the type of treatment applied to each flower was marked by a color-coded piece of yarn around the base of the treated flower. In 2014, color-coded enamel paint was dabbed on the tepals of treated flowers but was shown to be less effective than yarn as a marking tool because the painted tepals dry and fall off during fruit development. There was also concern that the paint could interfere with reproductive development. For self-crossing experiments, pollen from anthers of the test flower was extracted using a small paintbrush and dusted on the stigma of the test flower. For the out-crossing experiments, pollen was extracted from approximately 10 flowers from plants scattered throughout the population, mixed in a test tube, and a sample of the mixed pollen placed on the stigma of the treated flower using a small paint brush. Care was taken to

use the same technique in applying pollen to the stigma in both types of crosses. In addition, the height relative to the anthers, shape, color, and number of lobes were recorded for each stigma subjected to the hand-crossing tests. Fruit set from caged specimens was compared to reproductive success on uncaged plants that were accessible to pollination by natural pollinators. The uncaged flowers used as the control group were also marked with yarn and the date the flowers were marked was recorded. During the 2015 study, seeds were collected from fruits resulting from marked flowers and placed on native soil samples in sterile petri dishes and germinated to test viability. Seeds were given a 2 month window to germinate. Seeds that did not germinate after 2 months were subjected to a "pinch" test, in which seeds were pinched between the thumb and forefinger. If the seed collapsed easily with little pressure, the seed was considered inviable. For this study, seeds were considered viable if they either germinated or did not germinate but passed the "pinch" test. Seedlings that resulted from the viability tests will be returned to their site of origin.

An aniline blue fluorescent stain test (Dafni et al. 2005) was used to ascertain pollen tube growth on the stigma. Eighteen virgin flowers from 16 greenhouse plants and 10 virgin flowers from 5 field plants were hand pollinated using viable pollen representing either a self-pollination or cross-pollination. Of the 28 stigmas tested, 20 were self-crossed and 8 were out-crossed. In addition, virgin (caged) flowers (N=5) from the Kerr WMA site were collected to determine if self-pollination occurred naturally without the interference of hand-pollination. Pistils were excised and fixed in FPA solution (formalin 40%, concentrated propionic acid, 50% ethanol, 5:5:90 by volume) for 24 hours and then subsequently stored in 70% ethanol (Dafni et al. 2005). Pistils were treated with aniline blue using the procedure

outline by Dafni et al. 2005. Presence of yellow to yellow-green fluorescent pollen with pollen tubes indicated that the pollen germinated on the stigma.

RESULTS

Floral Phenology

Stigma Phenology and Receptivity

Prior observations of floral phenology were used to estimate flower age. The primary characteristics used were the color and position of the stigma lobes; secondary features included the condition of the anthers and tepals. Based on the images from the cameras, stigmas remained green and fisted for an average of 2 days and then became larger, either yellow or pink, and formed either a cup or a flat surface at maturity. Of the 72 mature stigmas observed during the 2015 hand-pollination tests, 59 (82%) had cupped stigma lobes, while in 13 (18%) flowers stigmas were flat. In addition, 58 (81%) stigmas were positioned above the anthers, while the other 14 (19%) were positioned even with the anthers.

Of the 37 stigmas collected to test the location and duration of receptivity, 5 were collected from buds, 13 were collected from young flowers, and 19 were collected from mature flowers. The peroxidase paper test showed no stain on the stigma of one bud with lobes of the other four buds showing very light staining on the ventral edges of only 1 or 2 lobes, indicating very low receptivity. Out of the 13 young flowers, 8 had light/partial staining on the ventral edges on as many as half of the lobes while the other 5 stigmas did not stain; these results also suggest no to little receptivity (Table 2). All of the 19 mature flowers developed dark staining primarily on the ventral edges and tips of each lobe, indicating higher receptivity. Figure 3 shows the difference in appearance and staining between young and mature flowers.

TABLE 2--Comparison of stigma receptivity between buds, young flowers, and mature flowers of the Tobusch fishhook cactus. Stained regions indicate areas of stigma receptivity.

Stigma Age	Number Tested	Number Stained	Stain Light/Dark	Stain Location
Bud	5	4	Light	Ventral edges of 1-2 lobes
Young	13	8	Light	Ventral edges on half or less of the lobes
Mature	19	19	Dark	Ventral edges and tips of all lobes

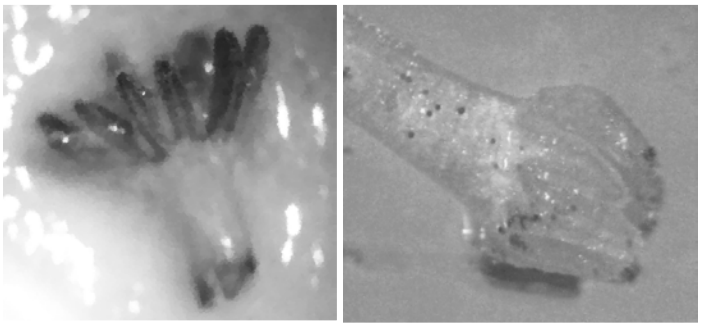

FIG. 3--Comparison between a mature (left) and a young (right) stigma from the Tobusch fishhook cactus. The dark coloration on the ventral edges and tips represent sites of stigma receptivity stained with Peroxtesmo esterase indicator papers (Dafni and Maués 1998).

Stamen Phenology and Pollen Viability

The outer stamens in Tobusch fishhook cactus flowers are attached along the sloping sides of the floral cup and continue to the floor of the cup such that the outer stamens are positioned higher than those in the middle. The flowers are protandrous with the anthers dehiscing before the bud opens, and the stigma displays low receptivity for one to two days after the flower has opened. Field observations and camera images showed that on days with honey bee visitation most of the pollen is removed from the flower during the first day of flowering. Three video sequences made from images taken on separate days (14 February,

15-16 March) showed that the stamens are thigmonastic, in that they move in response to stimuli. When stimulated by a floral visitor, the stamens move immediately inwards and towards the pistil. After departure of the visitor, the stamens retract to their original position approximately 10 seconds later.

In 2014, results from the MTT staining determined that pollen viability in young flowers ranged from 82-96% ($\bar{x}$= 92%) and decreased in mature flowers, ranging from 64-79% ($\bar{x}$= 68%). A permutational ANOVA (R Core Team 2013) indicated that there is a significant decline in pollen viability with flower age ($P<0.001$). In 2015, the average proportion of viable pollen in young flowers was approximately 33.9%, while the average proportion viable in mature flowers increased to about 50.8%. Variation in pollen viability ranged in young flowers from 21.0-56.0% with a median of 0.5 and mature flowers from 10.0-93.0% with a median of 0.3 (Fig. 4). The initial permutational ANOVA test showed a p-value close to 0.05. Due to concerns over a possible type II error, a permutational ANOVA (R Core Team 2013) with 1000 permutations for each subset was used to determine that approximately 35% of the time the test reported a significant difference ($P<0.05$) in pollen viability between groups.

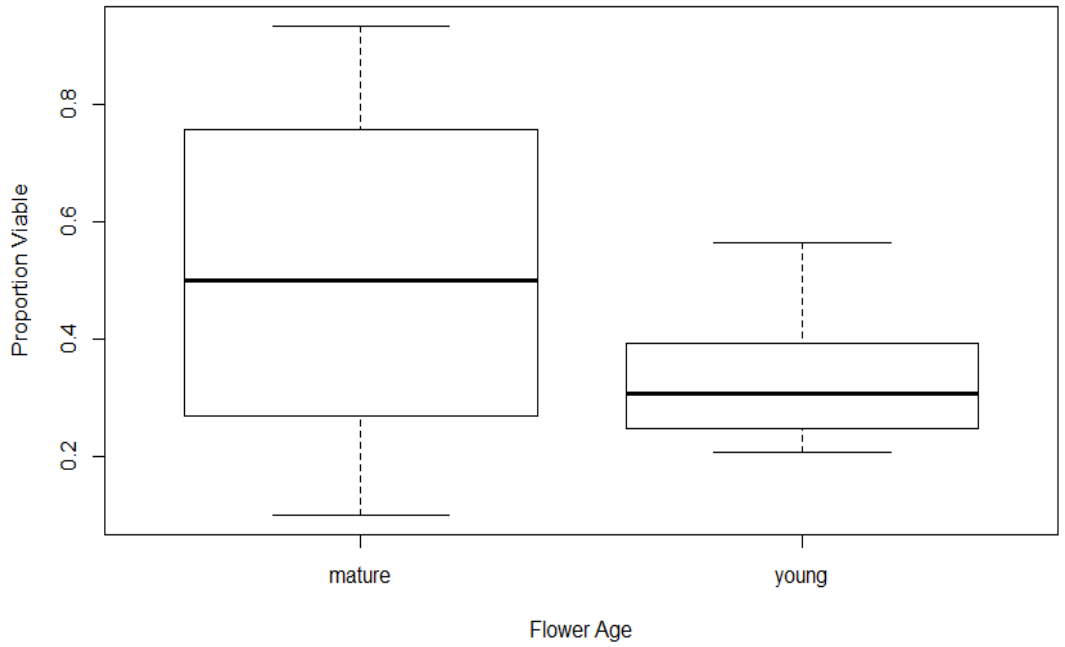

FIG. 4-- Boxplot of pollen viability proportions in young and mature flowers of the Tobusch fishhook cactus.

Nectar Study

In 2014, no saturation of the nectar wicks occurred in any of the flowers tested, and none of the flowers showed presence of sugars using the glucose test strips. Additionally, none of the 29 flowers tested with either 1:10,000 neutral red or the phenol/sulfuric acid method showed any indication of the presence of active floral nectaries or carbohydrates.

During the 2015 study, nectar tests were repeated to confirm that the lack of nectar in 2014 was not the result of persistent drought. No active floral nectaries or carbohydrates were detected in the 21 flowers treated with either 1:10,000 neutral red or the phenol/sulfuric acid method. The 2015 study corroborated the 2014 results in that no floral nectaries were identified and no nectar was present. However, the 6 samples of fluid collected from the

tubercle tips of greenhouse plants tested positive for the presence of glucose, confirming the substance as extrafloral nectar.

Pollen-Ovule Ratio

The nine flower buds used to calculate pollen-ovule (P/O) ratios showed the number of stamen per flower ranged from 204-470 ($\bar{x}$=336), and the number of pollen grains per flower ranged from 110,568-254,740 ($\bar{x}$=182,344) (Table 3). Ovule number per flower averaged 102 with a range from 62 to 128. P/O ratios ranged from 1,316.9-2,946.0 with a mean of 1,885.3.

TABLE 3--Pollen-ovule (P/O) ratios of Tobusch fishhook cactus flower buds (N=9) collected from the Kerr WMA.

Bud	Stamens	Total Number of Pollen Grains	Ovules	P/O Ratio
1	204	110,568	63	1,755.1
2	337	182,654	62	2,946.0
3	331	179,402	123	1,458.6
4	295	159,890	110	1,453.6
5	311	168,562	128	1,316.9
6	407	220,594	126	1,750.8
7	470	254,740	119	2,140.7
8	369	199,998	77	2,597.4
9	323	175,066	113	1,549.3

Flower Anthesis

The flower opening/closing times along with hourly temperature (°C) and cloud coverage for 12 days are listed in Appendix I. The temperatures associated with flower opening ranged from 17.5-21.6°C, with a mean temperature of 19.1°C. Flower closing temperatures ranged from 19.6-26.1°C with a mean of 22.9°C. The number of hours flowers stayed open varied from less than 1 hour on the 13th of February, which had a low of 5°C and

a high of 20.5°C ($\bar{x}$=14.4°C) to longer than 10 hours on the 29th of March, which ranged from 11.3-27.5°C ($\bar{x}$=21.8°C). Flowers did not open on the 15th of February and the 17th of March. On these dates, temperatures did not exceed 18.5°C and cloud cover remained primarily scattered to overcast. The logistic regression analysis indicated temperature and hour to have an effect on anthesis, while Julian day and cloud cover did not (Table 4).

TABLE 4--Logistic regression additive model used to predict factors affecting flower anthesis in the Tobusch fishhook cactus. (Jday = Julian day; Hour = Time of day; Temp = Temperature °C; SkyCLR, SkyOVC, SkySCT = Cloud cover estimate (CLR = clear; SCT = scattered (1-5 tenths sky cover); OVC = overcast).

Variable	Coefficient	SE	95% CI	P-value
Constant	-14.45	2.62	(-20.25, -9.88)	3.38E-08
Jday	-0.02	0.02	(-0.06, 0.02)	0.28
Hour	-0.29	0.12	(-0.54, -0.07)	0.02
Temp	0.97	0.18	(0.65, 1.36)	6.32E-08
SkyCLR	0.39	0.77	(-1.13, 1.92)	0.61
SkyOVC	1.16	0.83	(-0.43, 2.88)	0.16
SkySCT	-0.64	0.85	(-2.36, 1.01)	0.45

Evaluating Pollinator Effectiveness

Based on images, honey bees were the most common floral visitor with a total of 376 observations, while halictid bees were the second most common floral visitor with a total of 328 observations. Ants were the only other floral visitor observed with the cameras with a total of 214 observations, but 2 sulphur butterflies (Pieridae) and many small, red mites (Trombidiidae) were observed visiting flowers. Also, many of the flowers viewed under a dissecting microscope had thrips (Thripidae) in the stamen mass.

Of the 376 honey bee observations, the bees were vertical with the head down in the flower 317 times, which was interpreted as pollen collecting behavior. Of the 59 remaining observations, 26 showed honey bees attempting bud entry, while the other 33 observations showed honey bees either in mid-flight or resting on the flower, but not in contact with the stigma. Of the 372 honey bee in-flower observations, omitting the four in-flight observations, honey bees were in young flowers 61.8% (N=230) of the time, while 38.2% (N=142) observations occurred on mature flowers. Results from a binomial exact test (R Core Team 2013) indicated that honey bees are selecting young flowers over mature flowers ($P<0.001$). Of the 328 observations of halictid bees, 49 (14.9%) times the bee was on the stigma, while the other 279 (85.1%) observations showed halictids either among the stamens or on the tepals of the flower, but not in contact with the stigma. In addition, 96 (29.3%) observations showed halictids on young flowers whereas 232 (70.7%) showed halictids on mature flowers. Results from a binomial exact test (R Core Team 2013) indicated that halictid bees are selecting mature flowers over young flowers ($P<0.001$). Floral visitation time for honey bees ranged from 10 seconds to over 2 minutes with an average visitation time of approximately 30.7 seconds. Floral visitation time for halictid bees ranged from as short as 10 seconds to as long as 8 minutes with an average visitation time of approximately 2.1 minutes.

To determine the frequency of bee visitation throughout the season, the daily number of observations of open flowers containing either a honey bee or a halictid bee was compared to the total number of daily photos to give a daily percentage of floral visits (Table 5, Fig. 5). Honey bees were most frequent on the 14th of February (8.52%) with visitation declining after mid-March. Halictid bees were present as early as the 14th of February (0.63%) and became more frequent mid-March, with a peak frequency of 2.74% on March 16th.

TABLE 5--Frequency of daily floral visitors in Tobusch fishhook cactus flowers during the 2015 study period. (Number of Cameras = number of active cameras by date; HB = number of observations of honey bees by date; HA = number of observations of halictid bees by date; HB % = daily percentage of honey bee observations; HA % = daily percentage of halictid bee observations).

Date	Number of Cameras	HB	HA	Total Number of Observations	HB %	HA %
13-Feb	1	0	0	11	0.00	0.00
14-Feb	1	54	4	634	8.52	0.63
14-Mar	8	32	11	3,862	0.83	0.28
15-Mar	7	117	18	6,577	1.78	0.27
16-Mar	7	166	229	8,353	1.99	2.74
28-Mar	7	6	31	10,739	0.06	0.29
29-Mar	4	1	30	9,935	0.01	0.30
30-Mar	4	0	5	6,109	0.00	0.08
31-Mar	4	0	0	5,073	0.00	0.00
1-Apr	4	0	0	1,792	0.00	0.00

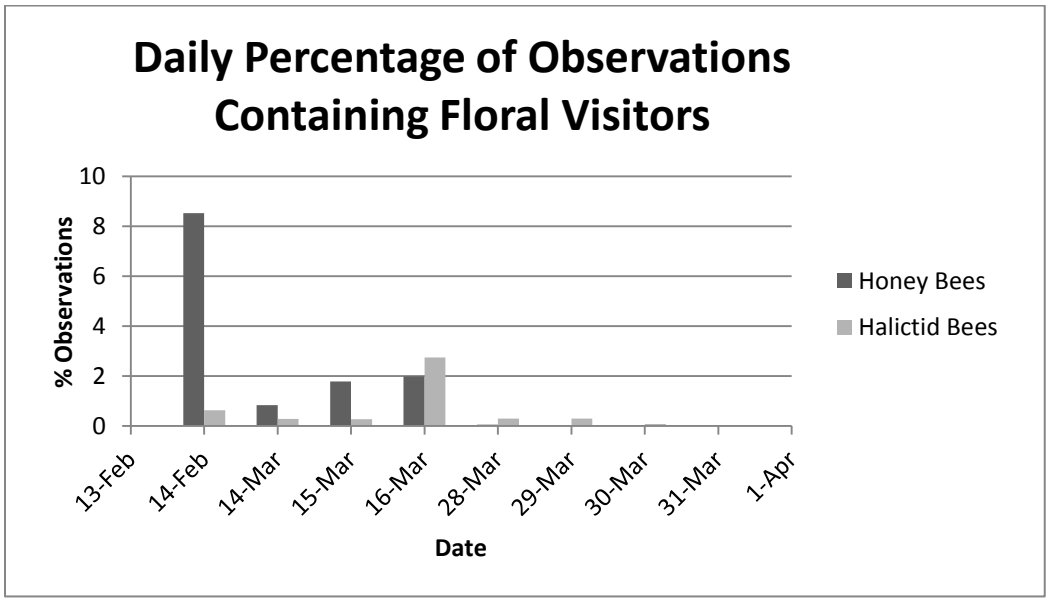

FIG. 5--Daily percentages of observations of honey bees and halictid bees on Tobusch fishhook cactus flowers during the 2015 study period.

Breeding System Investigation

During the 2014 season, a total of 24 flowers from 20 individual plants from the Kerr WMA were used in out-crossing experiments, while 15 flowers from the same 20 plants were used for self-crossing experiments. At the Junction site, 20 flowers from 21 plants were used for out-crossing, and 26 flowers from the same 21 plants were used for self-crossing. In late April the site was revisited for fruit set. Although the fruits were small and immature, the number of fruits was recorded in case conditions caused fruit loss prior to maturation. It was estimated that at the Kerr WMA site, 12 (50%) out-crosses and 4 (27%) self-crosses set fruit, while at the Junction site, 12 (60%) out-crosses and 8 (31%) self-crosses set fruit. A proportion test (R Core Team 2013) showed that at the beginning of the 2014 fruiting period, there was no statistically significant differences in fruit set between out-crosses and self-crosses at both the Kerr WMA (χ^2=1.225, df=1, P=0.268) and Junction (χ^2=2.831, df=1, P=0.092) sites. Both sites were visited again in mid-June to find that the fruits had already dehisced; therefore no seeds could be harvested to test viability.

During the 2015 season, 32 flowers from 26 plants from the Kerr WMA were used in out-crossing experiments, while 33 flowers from the same 26 plants were used for self-crossing tests. The control group consisted of 40 flowers from 36 plants. At the Junction site, 7 flowers from 9 plants were used for out-crossing, and 7 flowers from the same 9 plants were used for self-crossing. The control group consisted of 11 flowers all from different plants. Although the fruits were small and immature in late April, the number of fruits was recorded in case conditions caused fruit loss. The Kerr WMA had 22 (69%) outcrosses, 6 (18%) self-crosses, and 23 open-pollinated flowers (58%) set fruit (Table 6). At the Junction site, all flowers aborted except for one self-cross. Maturation of the fruits began to occur in

early-mid June. Another survey in mid-June showed that 14 (44%) out-crosses, 1 self-cross (3%), and 12 (30%) open-pollinated flowers continued to set fruit while the rest of the fruits aborted, including the one remaining self-cross at Junction. A pair-wise comparison using proportion tests (R Core Team 2013) of the June data, after adjusting p-values using Holm's method, determined that fruit set for out-crosses and open-pollinated flowers was statistically similar (χ^2=0.922, df=1, P_{adj}=0.337), but proportion fruit set from self-crosses was significantly lower when compared to out-crosses (χ^2=12.968, df=1, P_{adj}<0.001) and open-pollinated flowers (χ^2=7.238, df=1, P_{adj}=0.014). A final survey in early July showed that all fruits that remained during the June survey had set fruit to maturity.

TABLE 6--Comparison of fruit set between treatment (out-crosses, self-crosses) groups and the control (open-pollinations) group at both sites from surveys conducted in April, June, and July of the Tobusch fishhook cactus during the 2015 study period.

Treatment	**April Survey**		**June Survey**		**July Survey**	
	Kerr WMA	**Junction**	**Kerr WMA**	**Junction**	**Kerr WMA**	**Junction**
Out-crosses	69% (N=22/32)	0% (N=0/7)	44% (N=14/32)	0% (N=0/7)	44% (N=14/32)	0% (N=0/7)
Self-crosses	18% (N=6/33)	14% (N=1/7)	3% (N=1/33)	0% (N=0/7)	3% (N=1/33)	0% (N=0/7)
Open-pollinations	58% (N=23/40)	0% (N=0/11)	30% (N=12/33)	0% (N=0/11)	30% (N=12/33)	0% (N=0/11)

When the sites were visited in mid-June, most of the fruits had dehisced and the seeds dispersed. Seeds were able to be harvested from 3 out-crossed fruits, 1 self-crossed fruit, and 8 open-pollinated fruits. The site was revisited in early July to collect seeds from the remaining fruits, but the seeds had already dispersed. The harvested seeds were placed on

native soil in petri dishes and placed in an environmental chamber to compare seed viability between the treatments. Seeds remained in the germination chamber for two months. Seeds that did not germinate in the two-month period were subjected to the "pinch" test. The total number of seeds per fruit, the number germinated, the number of "pinch" test failures, and the percentage of viable seeds were recorded (Table 7). Seed viability for open-pollinations ranged from 75-100% ($\bar{x}$=85%), and seed viability for out-crosses ranged from 84.8-85.7% ($\bar{x}$=85.4%). The self-cross showed 80% seed viability. A permutational ANOVA (R Core Team 2013) was used to determine that there was no significant difference in seed viability between out-crossed and open-pollinated fruits (P>0.95). Since seeds were harvested from only one self-crossed flower, the self-crossed fruit was excluded in the permutational ANOVA test.

TABLE 7--Comparison of Tobusch fishhook cactus seed viability between out-crossed, self-crossed, and open-pollinated flowers.

ID	Total Seeds	Number Germinated	Number of Pinch Test Failures	% Viable
306 open	25	4	3	88.0
310 open	11	5	2	81.8
540 open	4	0	2	50.0
541 open	4	3	0	100.0
542 open	29	28	1	96.6
582 open	29	0	3	89.7
588 open	12	6	3	75.0
593 open	26	10	1	96.2
832 out	33	4	5	84.8
544 out	21	1	3	85.7
559 out	14	0	2	85.7
560 self	30	21	6	80.0

All 28 stigmas tested for pollen tube growth using aniline blue fluorescent staining (Dafni et al. 2005) had abundant pollen tubes throughout the lobes. There was no observable difference in the amount of pollen tube growth between stigmas treated with self- or cross-pollen. Since all 20 self-pollinated stigmas had germinated pollen, these results confirm that self pollen from the same flower will germinate on the stigma. In addition, the 5 virgin flowers collected to determine if self-pollination occurred naturally without the interference of hand-pollination showed many pollen tubes throughout the lobes, confirming that self-pollination occurs naturally.

DISCUSSION

Breeding System

The higher abortion rates of self-crossed fruits compared to out-crossed and open-pollinated fruits observed in this study during the 2015 reproductive season support the earlier results of Emmett's study (1995) and Zimmerman's 1981 investigation that the Tobusch fishhook cactus is primarily self-incompatible. Although differences in fruit set for the 2014 fruiting season were statistically insignificant, the survey was conducted when the fruits were small and immature and some fruits may have aborted later in development. Additionally, results from the stigma receptivity and pollen viability tests indicate that the flowers of the Tobusch fishhook cactus are protandrous, confirming Emmett's observation in his studies at different populations (1995). In this study, self-pollination was observed to occur naturally in this species, and self-pollen grains germinated on the stigma. This may have a negative effect on the success of cross-pollen due to obstructed access to the stigmatic surface and blockage of the style by self-pollen tubes (i.e. stigma clogging) (Doust and Doust 1988). Self-pollen germination may have implications on fruit and seed development since stigma pollen loads containing a mixture of self and cross pollen have been shown in many studies to reduce fruit and seed production in species with self-incompatibility systems (Bertin and Sullivan 1988, Lloyd 1992, Plitmann 1993, Cruzan and Barret 1996, Levin 1996, Stephenson et al. 2000). Although the "pinch" test for seed viability helps to establish the presence of an embryo, proportion seed viability reported in this study may be a high estimate due to the possibility that some seeds that passed the "pinch" test may not germinate.

Additionally, many species in the family Cactaceae have relatively low P/O ratios (<1,000) (Cruden 2000), which is thought to be an energy-saving strategy in stressful environments (Nobel and Bobich 2002). Although the P/O ratios observed in this study were slightly higher with an average of 1,885.3, the ratio is low when compared to entomophilous species of other plant families (5,000-100,000) and wind-pollinated flowers with P/O ratios ranging from 500,000- 3,000,000 (Nobel and Bobich 2002). Cruden (1977) proposed that P/O ratios are indicators of pollination effectiveness. In general, autogamous species will have lower P/O ratios than xenogamous species because autogamy has higher pollination efficiency. In addition, animal-pollinated species will have lower P/O ratios than wind-pollinated species because wind pollination is less efficient (Cruden 2000). The P/O ratios of the Tobusch fishhook cactus fall within the xenogamy range of the breeding systems outlined by Cruden (1977).

Pollinator Effectiveness

Since the Tobusch fishhook cactus is primarily self-incompatible and requires the transfer of pollen by floral visitors to reproduce, identifying the effective pollinators becomes critical to the conservation of this species. The observation from this study that honey bees (Apidae) and halictid bees (Halictidae) are the most frequent floral visitors is consistent with findings from previous studies (Emmett 1995, Lockwood 1995, and Reemts and Becraft 2013). Based on this study's observations of overall abundance and intrafloral behavior, honey bees were determined to be the most important and effective pollinators. Although halictid bees are frequent floral visitors and have relatively long visit durations, they were observed contacting the stigma only approximately 15% of the time. Therefore, many floral visits by halictid bees may not result in successful pollination. Additionally, the Tobusch

fishhook cactus was observed in this study to have thigmonastic stamens that move inward and towards the central part of the flower. The function of thigmonastic stamens has been hypothesized to increase the chance of stigma contact while the insect is foraging in *Opuntia* cacti by encouraging movement to the central part of the flower (Cota-Sánchez et al. 2013). However, since most flowers of the Tobusch fishhook cactus have stigmas situated above the anthers, it remains unclear if this mechanism increases stigma contact by halictid bees in this species.

In addition, this study suggests that honey bees have a predilection for young flowers, while in contrast, halictid bees have a propensity to select mature flowers. The greater amount of pollen in young flowers may explain the reason why honey bees visited young flowers significantly more than older flowers. In addition, non-viable pollen has lower amino acid content than viable pollen (Heslop-Harrison 2013). If pollen viability decreases with flower age, this could be another explanation as to why honey bees actively select young flowers. Furthermore, honey bees have been known to out-compete halictid bees through interference competition (Schaffer and Schaffer 1979) which may result in halictid bees avoiding young flowers that are subject to frequent honey bee visitation. Additionally, honey bee visitation in Tobusch flowers is most common in the early part of the season, especially February, and declines after mid-March. The decline in honey bee visitation correlates with the beginning of the flowering season for other plant species in the region.

Nectar Study

No intrafloral nectaries or the production of intrafloral nectar was observed throughout this study, indicating that pollen likely serves as the primary floral attractant. Although nectar production is common in Cactaceae, some species of cactus do not produce

nectar which may be an adaptation for water conservation (Grant and Connell 1979). Cruden (2000) observed that the P/O ratios of plants that provide only pollen as a reward are higher than those that also present nectar as a reward, which may explain the slightly higher P/O ratios of the Tobusch fishhook cactus, compared to some nectar-producing cacti species. Extrafloral nectaries are common in Cactaceae and have been reported in some species of *Sclerocactus* (Felger et al. 2014). Early reports suggest that extrafloral nectaries are used to attract ants which in return offer protection (Beattie 1985, Davidson and McKey 1993, Whitman 1994), which may explain why the Tobusch fishhook cactus produces extrafloral nectar during the fruiting period. However, based on Emmett's observations of seed collection by ants (*Forelius foetidus*) (Dolichoderinae) at all three sites (Emmett 1995), the extrafloral nectaries may serve some other function in this species.

Flower Anthesis

According to the results of the logistic regression analysis, the factors that have the greatest effect on flower anthesis are temperature and time of day. Although cloud cover likely effects flower anthesis by altering light levels, temperature plays a greater role according to the results of this study. On some taxa from previous reports, temperature was determined to be the main factor affecting floral anthesis. In a study on *Portulaca* plants (Doorn and Meeteren 2003), a rise in temperature in the absence of light was sufficient for flower anthesis, although light intensified the response. In contrast, exposure to light without a temperature change did not result in full anthesis. Other taxa in which flower anthesis mainly depends on temperature are *Ficaria*, *Galanthus*, *Tulipa*, and *Crocus* (Doorn and Meeteren 2003). Although this study suggests temperature is the main factor affecting anthesis, these results are preliminary since cloud cover is a rough estimate of light levels and

the analysis was limited to 12 days. Future studies should analyze the effects of light intensity and temperature on flower anthesis by testing the effects of increasing temperature in the absence of light.

Study Conclusion

Although self-incompatibility can effectively reduce the risk of inbreeding (Levin 1981), it may also prevent adequate reproduction in small, isolated populations (DeMauro 1993). Habitat fragmentation from the growing number of road, fences, power lines, and residential development in the Edward's Plateau region increases the risk of Tobusch fishhook cactus populations becoming more fragmented and isolated. Furthermore, the two populations used in this study rely primarily on honey bees for successful pollination, which may be a concern with the consecutive years of high honey bee mortality and colony population declines (Natural Research Council 2006).

The large variation in pollen viability observed in this study is likely the result, in part, of age variation in mature flowers and differences in pollen viability across individual plants. In future studies, pollen viability should be monitored through the life of the same flower. The average lifespan of an individual pollen grain needs to be determined in order to establish if pollen transferred to a young, unreceptive stigma will stay viable until the stigma matures. This will determine if insect visitations on young flowers, especially by honey bees, result in successful pollination. In addition, future studies should focus on the diversity of halictid visitors and analyze differences in foraging behavior and pollinator efficiency. Furthermore, additional studies are needed to determine the environmental conditions necessary for extrafloral nectar production and the role of extrafloral nectar during fruit set.

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APPENDIX I

Flower Anthesis Times- List of anthesis times, hourly temperature (°C), and hourly cloud coverage for 12 days during the February-April 2015 flowering season. “Open” times in bold do not represent the beginning of anthesis; the camera was moved that day to a flower already opened. (CLR= clear; SCT= scattered (1-5 tenths sky cover); BKN= broken (6-9 tenths sky cover); OVC= overcast). Temperature and cloud cover data was acquired from the [ERV] Kerrville Municipal station of the TX_ASOS network on the IEM website (IEM 2015).

	Time	Temp °C	Cloud Coverage	Open/Closed
13-Feb	7:15	5.0	CLR	-
	8:15	5.1	SCT	-
	9:15	8.7	CLR	-
	10:15	11.2	SCT	-
	11:15	14.8	CLR	-
	12:15	16.3	CLR	-
	13:15	18.1	CLR	-
	14:15	18.0	BKN	-
	15:15	19.0	SCT	-
	16:15	20.5	BKN	Open
	17:15	18.5	OVC	-
	18:15	17.0	OVC	-
	19:15	14.5	CLR	-
14-Feb	7:15	3.9	CLR	-
	8:15	5.7	CLR	-
	9:15	9.2	CLR	-
	10:15	14.8	CLR	-
	11:15	19.0	CLR	Open
	12:15	20.5	CLR	Open
	13:15	22.0	CLR	Open
	14:15	22.0	SCT	Open
	15:15	22.2	SCT	Open
	16:15	22.0	SCT	-
	17:15	20.3	SCT	-
	18:15	17.7	CLR	-
	19:15	16.2	CLR	-
15-Feb	7:15	13.6	BKN	-
	8:15	14.1	CLR	-
	9:15	14.8	CLR	-
	10:15	16.2	BKN	-
	11:15	16.3	SCT	-
	12:15	16.2	BKN	-

	13:15	16.1	OVC	-
	14:15	15.5	OVC	-
	15:15	17.7	SCT	-
	16:15	18.1	OVC	-
	17:15	18.5	SCT	-
	18:15	17.6	BKN	-
	19:15	17.0	SCT	-
14-Mar	7:15	10.6	CLR	-
	8:15	10.6	CLR	-
	9:15	14.8	CLR	-
	10:15	17.0	SCT	-
	11:15	18.7	SCT	-
	12:15	20.5	CLR	**Open**
	13:15	22.0	CLR	Open
	14:15	22.1	CLR	Open
	15:15	22.5	BKN	Open
	16:15	22.1	BKN	-
	17:15	23.0	SCT	-
	18:15	22.0	CLR	-
	19:15	20.5	SCT	-
15-Mar	7:15	7.1	CLR	-
	8:15	7.1	CLR	-
	9:15	9.2	CLR	-
	10:15	12.1	CLR	-
	11:15	18.0	CLR	-
	12:15	18.6	CLR	-
	13:15	19.6	CLR	Open
	14:15	20.1	CLR	Open
	15:15	20.5	CLR	Open
	16:15	22.0	CLR	Open
	17:15	22.0	CLR	Open
	18:15	21.8	CLR	Open
	19:15	19.6	CLR	Open
16-Mar	7:15	7.8	CLR	-
	8:15	8.2	BKN	-
	9:15	10.6	SCT	-
	10:15	14.6	BKN	-
	11:15	17.6	BKN	Open
	12:15	19.7	BKN	Open
	13:15	19.7	OVC	Open
	14:15	23.2	SCT	Open
	15:15	22.0	OVC	Open

	16:15	22.0	OVC	Open
	17:15	22.0	OVC	Open
	18:15	19.6	OVC	-
	19:15	17.6	OVC	-
17-Mar	7:15	15.0	OVC	-
	8:15	15.0	OVC	-
	9:15	15.7	OVC	-
	10:15	16.2	OVC	-
	11:15	16.2	OVC	-
	12:15	16.2	BKN	-
	13:15	17.2	BKN	-
	14:15	17.7	OVC	-
	15:15	17.6	OVC	-
	16:15	17.3	BKN	-
	17:15	17.6	OVC	-
	18:15	18.3	SCT	-
	19:15	19.0	BKN	-
28-Mar	7:15	5.0	CLR	-
	8:15	5.8	CLR	-
	9:15	14.8	CLR	-
	10:15	18.9	CLR	-
	11:15	22.0	CLR	-
	12:15	22.1	CLR	-
	13:15	24.7	CLR	**Open**
	14:15	25.5	CLR	Open
	15:15	26.1	CLR	Open
	16:15	26.5	CLR	Open
	17:15	27.1	CLR	Open
	18:15	26.1	CLR	Open
	19:15	24.7	CLR	-
29-Mar	7:15	11.3	CLR	-
	8:15	12.0	CLR	-
	9:15	15.0	CLR	-
	10:15	17.5	SCT	Open
	11:15	20.9	SCT	Open
	12:15	23.3	CLR	Open
	13:15	24.7	CLR	Open
	14:15	26.1	CLR	Open
	15:15	26.9	CLR	Open
	16:15	27.5	CLR	Open
	17:15	27.5	CLR	Open
	18:15	26.1	CLR	Open

	19:15	24.7	CLR	Open
30-Mar	7:15	16.3	OVC	-
	8:15	16.2	OVC	-
	9:15	16.2	OVC	-
	10:15	17.0	OVC	-
	11:15	17.6	OVC	Open
	12:15	18.0	OVC	Open
	13:15	20.0	OVC	Open
	14:15	22.0	OVC	Open
	15:15	23.3	SCT	Open
	16:15	24.7	CLR	Open
	17:15	24.7	SCT	Open
	18:15	24.7	SCT	Open
	19:15	22.1	CLR	Open
31-Mar	7:15	17.1	SCT	-
	8:15	17.2	BKN	-
	9:15	17.6	OVC	-
	10:15	17.8	OVC	-
	11:15	19.0	OVC	Open
	12:15	21.9	BKN	Open
	13:15	22.0	SCT	Open
	14:15	22.6	CLR	Open
	15:15	23.3	BKN	Open
	16:15	23.2	OVC	Open
	17:15	23.4	CLR	Open
	18:15	22.1	SCT	-
	19:15	22.0	SCT	-
1-Apr	7:15	17.0	BKN	-
	8:15	17.6	SCT	-
	9:15	19.0	BKN	-
	10:15	20.5	BKN	-
	11:15	20.1	BKN	-
	12:15	18.3	OVC	-
	13:15	21.6	BKN	Open
	14:15	23.1	BKN	Open
	15:15	25.3	SCT	Open
	16:15	26.9	SCT	Open
	17:15	26.1	SCT	Open
	18:15	26.1	SCT	Open
	19:15	24.7	CLR	-